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Lipid-Mediated Light Activation of a Mechanosensitive Channel of Large Conductance

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This paper describes the reversible activation of a mechanosensitive channel via a light-sensitive lipid mimic. For these experiments, the mechanosensitive channel of large conductance (MscL) protein from *Lactococcus lactis* and *Escherichia coli* was reconstituted in lipid bilayers composed of 80 mol % 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and 20 mol % di-(5-[4-(4-butylphenyl)azo]phenoxy)pentyl)phosphate (4-Azo-5P). Light-induced isomerization of the azobenzene moiety of 4-Azo-5P from trans to cis was used to activate MscL.

The mechanosensitive channel of large conductance (MscL) serves as a pressure relief valve that protects bacteria against severe osmotic downshifts. Equivalent systems for mechanosensation are also present in higher organisms. The determination of the crystal structure of MscL from *Mycobacterium tuberculosis*¹ and the large amount of biochemical data for the homologous protein from *Escherichia coli*² has made MscL the paradigm in the research on membrane-mediated mechanosensation. MscL opens at membrane tensions close to the lytic limit of the bilayer. Changes in the lateral tension in the membrane, asymmetric bending, and membrane thinning are thought to be the main driving forces for channel opening.^{3–5} Although asymmetric bending can be elicited by applying a pressure across the membrane in patch clamp experiments, changing of the other membrane parameters requires other approaches of which reconstitution of MscL in different lipid mixtures and comparison of the channel properties in separate experiments is the easiest and most accurate. Here, we present the reconstitution of MscL in lipid bilayers with properties that can be altered via light-induced trans–cis isomerization of a lipid mimic (Figure 1). The trans-to-cis and cis-to-trans isomerizations result in a reversible increase and decrease, respectively, of the activity of the channel protein.

The MscL proteins from *E. coli* and *Lactococcus lactis* were modified with a carboxyl-terminal 6-histidine tag and amplified in *E. coli* PB104, using the pB10b expression vector.⁶ Membranes isolated from the cells were solubilized with Triton X-100, and the proteins were purified by nickel affinity chromatography. The purified protein was reconstituted, at a 1:20 000 channel-to-lipid molar ratio,

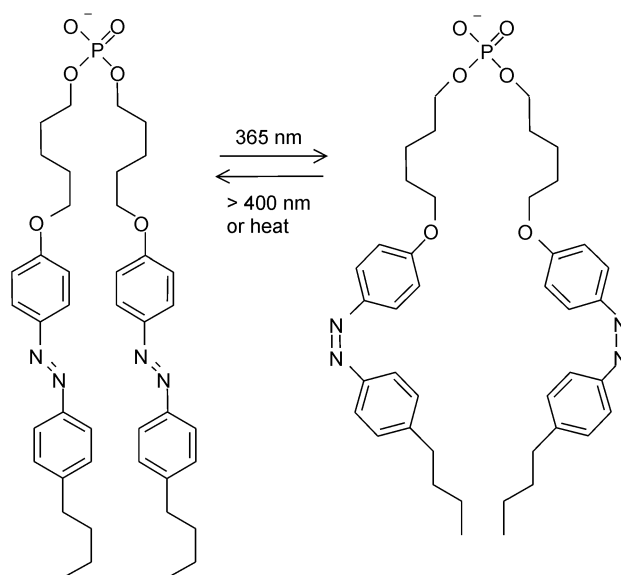


Figure 1. 4-Azo-5P structure in the trans (left) and cis (right) conformation. Switching between these conformations is elicited by illuminating at 365 nm (from trans to cis) or at >400 nm (from cis to trans).

into lipid bilayers containing 80 mol % 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) plus 20 mol % sodium di-(5-[4-(4-butylphenyl)azo]phenoxy)pentyl)phosphate (4-Azo-5P; Figure 1)⁷ or 20 mol % 1,2-dioleoyl-*sn*-glycero-3-phosphatidylserine (DOPS). As a control, liposomes without MscL were prepared. Subsequently, the (proteo)liposomes were dehydrated on glass cover slides coated with indium tin oxide (ITO) and then rehydrated in a flow chamber with 300 μ L of 0.25 mM K–N-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (Hepes), pH 7.2, 10 mM KCl, and 2 mM MgCl₂ plus 320 mM sucrose; the sucrose was used to make the rehydration buffer equimolar to the buffer for patch clamp analysis (5 mM Hepes, pH 7.2, 200 mM KCl, 40 mM MgCl₂). The flow chamber was closed with two ITO-coated glass slides, one of which contained the dried proteoliposomes. A voltage

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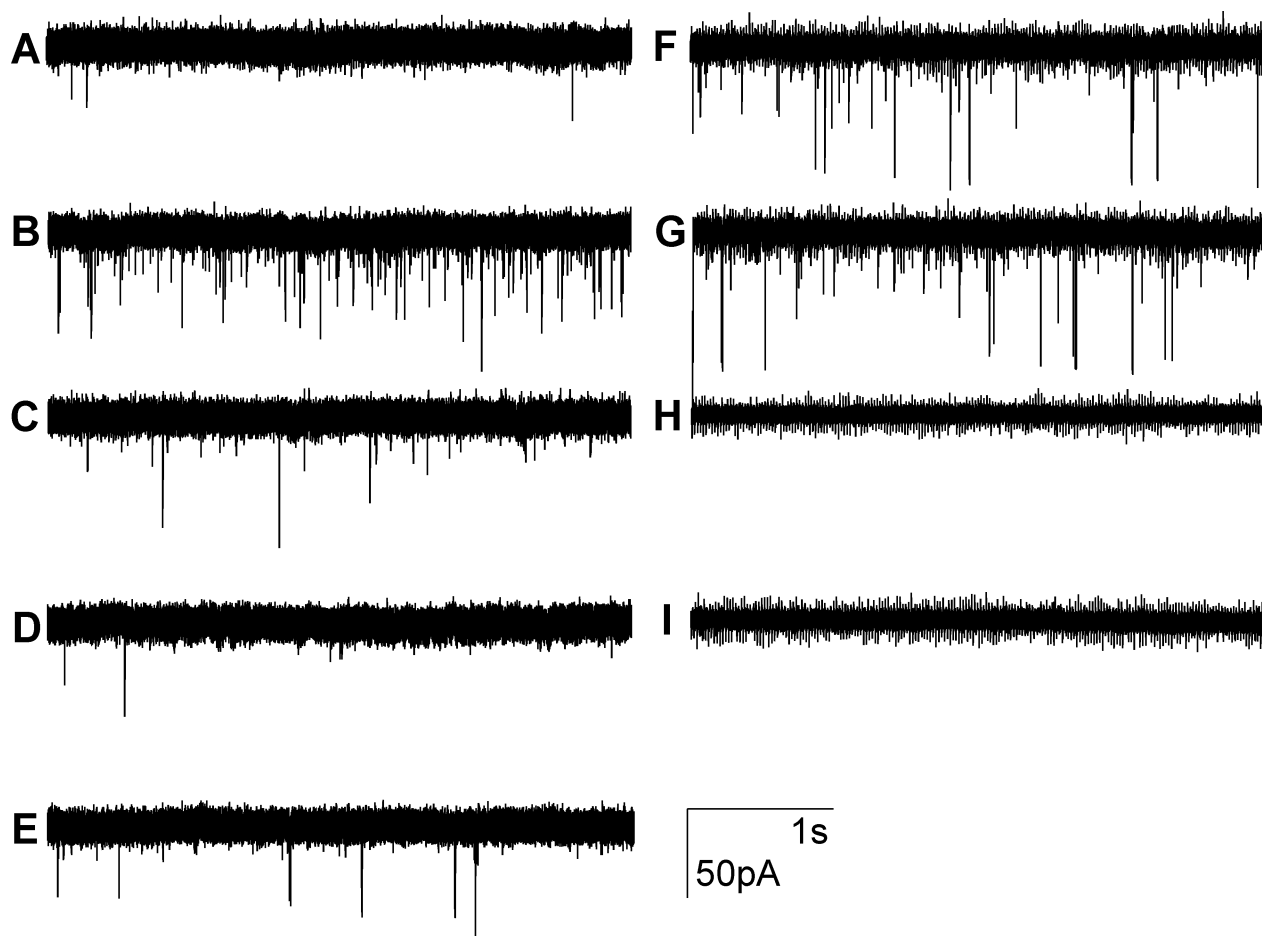


Figure 2. Channel activity of a single patch composed of DOPC: 4-Azo-5P (4:1 mol/mol), containing the *L. lactis* MscL channel. A, Before illumination of the sample (4-Azo-5P in trans conformation); B, after 30 s of illumination at 365 nm (4-Azo-5P in cis conformation); C, after a subsequent 60 s of illumination at 436 nm (4-Azo-5P back in trans conformation). D and E, Channel activity of a patch of DOPC:4-Azo-5P (4:1 mol/mol), containing the *L. lactis* MscL without illumination (D was recorded shortly after seal formation, E after 60 s). F and G, Channel activity of a patch of DOPC:DOPS (4:1 mol/mol), containing the *L. lactis* MscL before illumination (as soon as the seal had formed, F) and after 30 s of illumination at 365 nm (G). H and I, Activity of a patch of DOPC:4-Azo-5P (4:1 mol/mol) without channels (H was recorded shortly after seal formation, I after 30 s of illumination at 365 nm). All patches were clamped at -20 mV and a pipet pressure of around -10 mmHg. A continuous trace of MscL activity in a patch composed of DOPC:4-Azo-5P (4:1 mol %) with the light off and light on at 365 and 436 nm is shown in Supporting Information, section 2.

of 1.2 V at 10 Hz was applied for at least 3 h through electrodes sealed on the glass plates. The resulting giant unilamellar vesicles (GUVs), $5\text{--}50\text{ }\mu\text{m}$ in diameter, were used in patch clamp experiments.

After forming a giga-ohm seal by suction through a patch clamp electrode, the membrane patch was excised from the respective GUV and analyzed for the presence of channel activity. If channels were observed, the patch was clamped at a negative pressure between -5 mmHg and -15 mmHg. At that point, the light was switched off except for a Hg lamp (Oriel 180W) to illuminate the sample using a 365-nm mercury line filter with a bandwidth of 10 nm (for details see Supporting Information, section 1). This resulted in switching of the azobenzene moiety of 4-Azo-5P from the trans to the cis conformation.^{7,8} An increase in channel openings upon 4-Azo-5P lipid switching at constant pressure was observed (Figure 2A,B). Subsequent illumination with the same light source, but using a mercury line filter at 436 nm (bandwidth 10 nm), shifted the 4-Azo-5P back to the trans conformation⁸ and the channel activity decreased as shown in Figure 2C. The channel activities shown in Figure 2 correspond to

several substates (ca. 0.8 nS and 1.5 nS) and full openings at about 2.2 nS, which are typical for MscL from *L. lactis*.

After 90 s of illumination at 365 nm, 73% of the 4-Azo-5P lipid is in the cis conformation⁸ and the channel activity was increased about four times ($n = 13$; Figure 3). At the applied pressures (generally around -10 mmHg), it was not possible to activate all channels in the patch with the light-dependent switch of the lipids. The whole cycle of lipid switching and channel activation relaxation in a single patch could be repeated at least five times. After 50 s of illumination at 436 nm, when 89% of the *cis*-4-Azo-5P lipid is back in the trans position,⁸ the channel activity (after one cycle of switching) was about 1.3 times higher than at the start of the experiment (compare Figure 2A,C; Figure 3). This is probably caused by migration of the lipid patch inside the pipet (Supporting Information, section 3), but the small fraction (11%) of 4-Azo-5P still in the cis conformation may also have contributed to the increased open probability. A control experiment, in which the channel activity was monitored in the dark, with 4-Azo-5P lipids kept in the trans conformation for the duration of the experiment (3 min), is shown in Figure 2D,E. In 21 independent experiments without lipid switching, a small increase in channel activity was observed during the first

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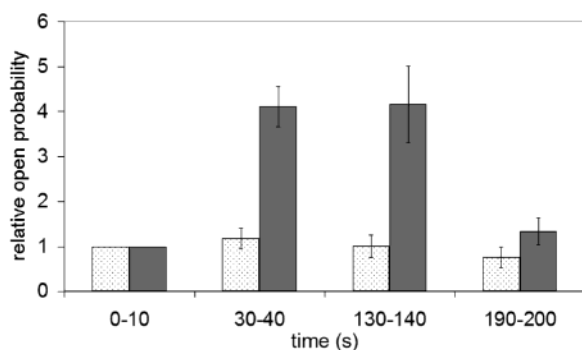


Figure 3. Quantification of the increase in open probability of MscL upon trans to cis isomerization of 4-Azo-5P. The open probability from 0 to 10 s was indexed at 1. The bars indicate the mean relative open probability in patches without switching (dotted bars) and with switching (gray bars); 30–40 s and 130–140 s of illumination at 365 nm and subsequent 50 s illumination at 436 nm (190–200 s). Error bars indicate standard error of the mean.

2 min. In a second control experiment, the 4-Azo-5P lipid was replaced by DOPS (Figure 2F,G). In four independent patches, the channel activity, quantified as above, no significant increase in channel activity was observed (Supporting Information, section 1, Table 1). Finally, empty liposomes containing 20 mol % 4-Azo-5P showed no change in electrical signal upon illumination at 365 nm (Figure 2H,I).

During the experiments, the position of the patch in the pipet was followed with a Nikon DXM charge-coupled device camera (Supporting Information, section 3). With the current camera setup, it was not possible to take pictures of the patch in the cis conformation because this required light of longer wavelengths, which caused switching back to the trans conformation. The increase in channel activity shown in Figure 2B must be the result from the light-dependent switching of 4-Azo-5P from the trans to the cis conformation, and this activation was entirely reversible. Because 4-Azo-5P is present in both leaflets of the patch membrane, it is not likely that activation is caused by a change in curvature strain (or elastic stress) of one leaflet relative to the other as is the case when amphipaths such as LysoPC or tetracain are used.³ This means that the channel is activated by a more subtle change in the overall lateral pressure profile or by membrane thinning. Molecular dynamics simulations (Supporting Information, section 4) showed that the shape of 4-Azo-5P molecules in a DOPC bilayer that is adopted

is different for the trans and the cis conformations. The mass distribution across the bilayer due to the 4-Azo-5P molecules is wider and more homogeneous for the trans conformation than for the cis conformation. Such changes probably affect the lateral pressure profile but are difficult to quantify. More elongated molecules such as trans 4-Azo-5P are presumably more compatible with the chain packing in the DOPC bilayer, leading to a larger bilayer thickness and a smaller bilayer area. This could lead to a larger compressibility of the bilayer; that is, it would require more energy to stretch the bilayer to the same extent in the presence of trans 4-Azo-5P than in the presence of cis 4-Azo-5P.

Differential scanning calorimetry studies on vesicles formed from 75 mol % 4-(dihexadecylmethyl)-1-methylpyridinium chloride and 25 mol % 4-Azo-5P showed that the main phase transition temperature decreased from 28 to 17 °C upon trans–cis isomerization.⁹ Similarly, deuterium NMR experiments on POPC-*d*₂ bilayers upon incorporation of 20 mol % 4-Azo-5P indicated that the acyl chain order decreased when the molecule was switched from the trans to the cis form,⁹ changes that correlate with the molecular dynamics simulations.

In summary, we have shown that we can reversibly activate MscL from both *L. lactis* and *E. coli* (not presented) by switching the 4-Azo-5P fraction (20 mol %) of the membrane from the trans to the cis conformation. Switching from trans to cis probably causes a change in the lateral pressure profile, resulting from a change in collisional pressure along the acyl chains of the lipid, and some thinning of the membrane, but elucidation of the mechanism leading to MscL opening requires more extensive molecular dynamics simulations. Importantly, the rapid and reversible alteration of the lipid bilayer via trans–cis isomerization of lipids allows monitoring of the effect of membrane perturbation on the conformational states of membrane proteins.

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Supporting Information Available: Experimental setup and data analysis and details for the continuous trace of channel activity under switching conditions, movement of the patch inside the pipet, and molecular dynamics simulations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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